

**Claim Listing:** This listing of claims will replace all prior versions and listings of claims in the application:

1. (original) A method for analyzing the organismic complexity of a sample, comprising:

- a) providing a sample containing one or more organisms;
- b) isolating the DNA from the organisms in the sample;
- c) contacting the DNA with a fragmenting enzyme, said fragmenting enzyme being a type II restriction endonuclease, under conditions appropriate for substantially complete digestion of the DNA thereby generating a plurality of DNA fragment species, each having complementary cohesive termini;
- d) incubating the DNA fragment species of step c) with a molar excess of a capture adapter, the capture adapter being a substantially duplex DNA having a portion which is covalently modified with a first member of a specific binding pair and also having one cohesive end compatible with the cohesive termini generated by the fragmenting enzyme of step c), under conditions appropriate for ligating the capture adapter to each of the complementary cohesive termini of the DNA

- 20            fragment species, thereby generating a plurality of  
             ligation products;
- e)    contacting the ligation products of step d) with an  
             anchoring enzyme under conditions for substantially  
             complete digestion of the ligation products, said  
             anchoring enzyme being a restriction endonuclease  
             having a high probability of cleaving a substantial  
             number of DNA fragment species generated in step c) at  
             least one time, thereby generating a plurality of  
             digestion products which have one cohesive terminus  
30            generated by the anchoring enzyme and a portion that  
             is covalently modified with a first member of the  
             specific binding pair;
- f)    capturing the digestion products of step e) by  
             contacting the digestion products with a solid support  
             having an attached second member of the specific  
             binding pair;
- g)    incubating the solid support and captured digestion  
             products of step f) with a molar excess of a duplex  
             linker having a type IIS restriction enzyme  
40            recognition sequence and one cohesive terminus  
             compatible with termini generated by the anchoring  
             enzyme of step e), under conditions appropriate for

ligating one duplex linker to the cohesive termini of the captured digestion products, thereby ligating a recognition sequence for a type IIS restriction enzyme to the captured digestion products;

- h) incubating the ligation product of step g) with the type IIS restriction enzyme, under conditions appropriate for substantially complete digestion

50 thereby releasing the duplex linkers, each having an appended signature tag;

- i) recovering the released duplex linkers and appended signature tags;

- j) incubating the recovered linkers and tags of step i) with a molar excess of an amplification adapter, the amplification adapter having one terminus compatible with the termini of the appended signature tags, the incubation being carried out under conditions

60 appropriate for ligating one amplification adapter to each appended signature tag;

- k) recovering the ligation products of step j);

- l) determining the nucleotide sequence of a statistically significant number of appended signature tags to generate a listing of signature tags; and,

m) relating the listing of signature tags of step l) to DNA sequences in databases to determine the variety and relative numbers of organisms originally present in the sample thereby analyzing the organismic complexity of the sample.

2. (original) The method of Claim 1 wherein the amplification adapter is characterized by having a restriction enzyme recognition site specific for the anchoring enzyme of step e) and which is located near the signature tag-compatible terminus.

3. (original) The method of Claim 1, further comprising:

n) amplifying the ligation products of step k) with a pair of primers comprising a first primer specific for the duplex linker and a second primer specific for the amplification adapter, wherein each primer is labeled with a first member of a second specific binding pair;

o) incubating the amplification products of step n) with the anchoring enzyme under conditions appropriate for complete digestion of the amplification products

thereby generating a digestion product mixture of end fragment digestion products and tag fragment products;

p) capturing the end fragment digestion products of step

o) by contacting the digestion product with a solid

support with a solid support having an attached second member of said second specific binding pair thereby leaving the tag fragment products in solution;

- q) isolating the tag fragment products from step p);
- r) ligating the isolated tag fragments of step q) to form concatemers;
- 20 s) isolating concatemers of sufficient length;
- t) cloning the concatemers of step s) in a plasmid vector to form concatemer constructs;
- u) transforming host cells with the concatemer constructs of step t);
- v) separately culturing individual transformed host cells of step u);
- w) separately isolating the concatemer constructs from the cultured cells of step v);
- x) determining the sequences of a statistically
- 30 significant number of concatemers of the isolated concatemer constructs of step w) to generate the listing of signature tags of step 1).

4. (original) The method of Claim 1 further comprising:

- n) diluting the ligation products of step k), as needed, to generate a solution containing two or fewer

individual ligation product members in a specific volume;

- o) separately amplifying the individual members present in the specific volume of step n) with a pair of primers, one specific for the duplex linker of step g) and the other specific for the amplification adapter of step j);
- p) sequencing a statistically significant number of the amplified members of step o) to generate the listing of signature tags of step l).

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5. (original) The method of Claim 1 wherein the solid support and captured digestion products of step f) are incubated with anchoring enzyme under conditions to ensure substantially complete digestion prior to the ligation of step g).
6. (original) The method of Claim 4 wherein the recovered ligation products of step k) are amplified with a pair of primers, one specific for the duplex linker of step g) and the other specific for the amplification adapter of step j), prior to the dilution of step n).
7. (original) The method of Claim 1 wherein the anchoring enzyme is a four-base cutter.

8. (original) The method of Claim 7 wherein the four-base cutter is selected from the group consisting of *NlaIII*, *DpnII*, *MboI*, *Tsp509I*, *MseI* and *Sau3AI*.
9. (original) The method of Claim 1 wherein the type IIS restriction enzyme is *MmeI*.
10. (original) The method of Claim 1 wherein the duplex linker is modified with a first member of a second specific binding pair and wherein the released duplex linkers and appended signature tags are recovered in step i) by contacting the released duplex linkers and appended signature tags with a second solid support having covalently attached a second member of the second specific binding pair.
11. (original) The method of Claim 1 wherein the specific binding pair is selected from the group consisting of biotin/streptavidin, antigen/antibody, sugar/lectin, apoenzyme/cofactor, hormone/receptor, enzyme/inhibitor, and complementary homopolymeric oligonucleotides.
12. (original) The method of Claim 1 wherein the solid support is selected from the group consisting of magnetic beads, glass beads, filter membranes, filter papers and polymeric beads.

13. (original) The method of Claim 3 wherein the second specific binding pair is identical to the specific binding pair of Claim 1.
14. (original) The method of Claim 3 wherein the second specific binding pair is different from the specific binding pair of Claim 1.
15. (original) The method of Claim 4 wherein the sequencing in step p) is performed using capillary gel electrophoresis.
16. (original) The method of Claim 4 wherein the dilution of step n) generates a solution having one or fewer individual product members in the specific volume.
17. (original) The method of Claim 16 wherein the sequencing is performed by a method selected from the group consisting of pyrosequencing and capillary gel electrophoresis.
18. (original) The method of Claim 1 wherein the sample is an environmental sample.
19. cancelled
20. (original) The method of Claim 1 wherein the sample is a biological specimen.
- 21 - 94. cancelled



95. (new) The method according to Claim 1 wherein:

- a) the anchoring enzyme is a four-base cutter;
- b) the type IIS restriction enzyme is *MmeI*;
- c) the specific binding pair is selected from the group consisting of biotin/streptavidin, antigen/antibody, sugar/lectin, apoenzyme/cofactor, hormone/receptor, enzyme/inhibitor, and complementary homopolymeric oligonucleotides;
- d) the solid support is selected from the group consisting of magnetic beads, glass beads, filter membranes, filter papers and polymeric beads; and
- e) the nucleotide sequence is determined by either capillary gel electrophoresis or pyrosequencing.

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